

Atty. Docket No.: P65141US0
Serial No.: 09/508,095

- collecting fractions;
- adjusting the fractions to a salt content of < 25 mM by dialysis or reverse phase HPLC for performing activity tests;
- culturing *Bifidobacterium bifidum* and *E. coli* in the presence of the fractions and selecting fractions which meet the requirement:

$$\frac{\text{BW} - \text{B0}}{\text{EW} - \text{E0}} \geq 0.15 \text{ (bifidogenic)}$$

wherein

- BW represents the germ count obtained upon 16 hours of incubation of *Bifidobacterium bifidum* in 50% Elliker broth in the presence of the peptides in a concentration of 200 µg/ml;
- B0 represents the germ count obtained in the control incubation without the peptides;
- EW represents the germ count obtained upon 16 hours of incubation of *E. coli* in 3 g/l tryptic soy broth in the presence of the peptides in a concentration of 200 µg/ml;
- E0 represents the germ count obtained in the control incubation without the peptides; and
- isolation of the peptides contained in this fraction.

14. A peptide having amino acid sequence

R₁-EQLLRLKK-R₂ (SEQ ID NO: 1), R₁-YLEQLLRLKKY-R₂ (SEQ ID NO: 2),
R₁-NRQRNILR-R₂ (SEQ ID NO: 3), R₁-YMNGMNRQRNILR-R (SEQ ID NO: 4),
R₁-FQWQRNMRK-R₂ (SEQ ID NO: 5), R₁-HTGLRRTA-R₂ (SEQ ID NO: 6),
R₁-FTAIQNLRK-R₂ (SEQ ID NO: 7), R₁-EVAARARVVW-R₂ (SEQ ID NO: 8),

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R₁-WQRNMRKV-R₂ (SEQ ID NO: 9), R₁-LARTLKRLK-R₂ (SEQ ID NO: 10),
R₁-YKQKVEKV-R₂ (SEQ ID NO: 11), R₁-LVRYTKKV-R₂ (SEQ ID NO: 12),
R₁-KYLYEIARR-R₂ (SEQ ID NO: 13), R₁-ARRARVWWCAVG-R₂ (SEQ ID NO: 14),
R₁-ARRARVWWCAVGE-R₂ (SEQ ID NO: 16), R₃-CIAL-R₄ (SEQ ID NO: 15)
R₃-CIAL-R₄ (SEQ ID NO: 15)

R₁-YQRRPAIAINNPYVPRTYYANPAVVRPHAQIPQRQYLPNSHPPTVVRPNLHPSF-R₂,
(SEQ ID NO: 17)

R₁-GRRRSVQWCTVSQPEATKFCWQRNMRVRGPPVSCIKRDSPICQCIQA-R₂
(SEQ ID NO: 18),

R₁-GRRRSVQWCAVSQPEATKCFWQRNMRKVRGPPVSCIKRDSPICQCIQA-R₂,
(SEQ ID NO: 19),

R₁-GRRRSVQWCAVSQPEATKCFWQRNMRKVRGPPVSCIKRDSPICQCIQA-R,
(SEQ ID NO: 20),

R₁-VYQHQKAMPKPWIQPKTKVIPYVRYL-R₂ (SEQ ID NO: 21),
R₁-ARRARVWWAAVG-R₂
(SEQ ID NO: 22),

R₁-CAVGGGCIAL-R₂ (SEQ ID NO: 23),

R₁-RHTRKYWCRQGARGGCITL-R₂ (SEQ ID NO: 24),

wherein

R₁, R₂, R₃, and R₄ are optionally present,
R₁, R₂ independently represents an amino acid or a peptide containing up to 100 amino acids, and
R₂, R₄ independently represents CONH₂, an amino acid, or a peptide containing up to 100 amino acids;

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the peptide modified at the N-terminus by amidation, acetylation, sulfation, phosphorylation, glycosylation, or oxidation having bifidogenic properties, or a fragment thereof having bifidogenic properties.

15. The peptide of claim 6 having the amino acid sequence SEQ ID NO: 8, SEQ ID NO: 14, SEQ ID NO: 15, or $R_1\text{-ARRARVVWCAVG-}R_2$ (SEQ ID NO: 14)
 $R_3\text{-CIAL-}R_4$ (SEQ ID NO: 15).

16. A method of obtaining peptides comprising the steps of:

- adding proteases to cow's milk, or human milk, followed by incubation for two hours;
- centrifugation to remove milk fat;
- acidification to a pH of 2.0 with strong acids;
- removing the precipitated proteins;
- application of at least one reverse phase HPLC step;
- application of a cation-exchange HPLC step;
- collecting fractions;
- adjusting the fractions to a salt content of < 25 mM by dialysis or reverse phase HPLC for performing activity tests;
- culturing *Bifidobacterium bifidum* and *E. coli* in the presence of the fractions and selecting fractions which meet the requirement:

$$\frac{\text{BW}}{\text{B0}} - \frac{\text{EW}}{\text{E0}} \geq 0.15 \text{ (bifidogenic)}$$

wherein

- BW represents the germ count obtained upon 16 hours of incubation of *Bifidobacterium bifidum* in 50% Elliker broth in the presence of the peptides in a concentration of 200 $\mu\text{g/ml}$;